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Patent

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

CLAIMS	Title: SCREENING FOR LIGAND BINDING AT SPECIFIC TARGET SITES	
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LISTING OF CLAIMS:

1. (Currently Amended) A method for screening of compounds for binding differentiation at various drug target binding sites, for use with a device measuring the enthalpy of reaction for such binding, comprising:

merging not less than one test ligand with not less than one target compound at not less than one first location on the device measuring enthalpy of reaction, wherein said target compound includes not less than one binding site of interest and not less than one binding site to be avoided;

merging not less than one test ligand with not less than one target compound in the presence of at least one first blocking agent at not less than one second location on the device measuring enthalpy of reaction, wherein said target compound includes not less than one binding site of interest and not less than one binding site to be avoided;

detecting a first heat of reaction for said merged not less than one test ligand and said not less than one target compound at said first location;

detecting a second heat of reaction for said merged not less than one test ligand with said not less than one target compound in the presence of a blocking agent at said second location; ~~and~~

comparing said first and second heats of reaction; and

determining a binding operation has occurred at the binding site of interest

of the first location when the comparing step finds the first heat of reaction is greater than the second heat of reaction.

2. (Original) The method for screening of compounds according to claim 1, wherein said at least one first blocking agent comprises a site-specific agent.

3. (Original) The method for screening of compounds according to claim 1, wherein said at least one first blocking agent comprises an excess of an enzyme specific substrate.

4. (Original) The method for screening of compounds according to claim 1, wherein said not less than one target compound comprises an enzyme target solution.

5. (Original) The method for screening of compounds according to claim 1, wherein said target compound and said test ligand at said not less than one first location are in the presence of at least one second blocking agent.

6. (Original) The method for screening of compounds according to claim 5, wherein said at least one second blocking agent comprises a cofactor solution and said at least one first blocking agent comprises an enzyme specific substrate solution.

7. (Original) The method for screening of compounds according to claim 5, wherein said at least one first blocking agent comprises a plurality of differing blocking agents.

8. (Original) The method for screening of compounds according to claim 7, wherein said plurality of differing blocking agents comprise an enzyme specific substrate solution and a regulatory agent solution.

9. (Original) The method for screening of compounds according to claim 7, wherein said plurality of differing blocking agents comprise an excess of enzyme specific substrate and a non-hydrolyzable nucleotide phosphate analog solution.

10. (Original) The method for screening of compounds according to claim 5, wherein said target compound comprises not less than one kinase target.

11. (Original) The method for screening of compounds according to claim 10, wherein said not less than one second blocking agent comprises an excess of ATP.

12. (Original) The method for screening of compounds according to claim 10, wherein said not less than one first blocking agent comprises a kinase specific substrate.

13. (Original) The method for screening of compounds according to claim 5, wherein said at least one second blocking agent comprises a plurality of differing blocking agents.

14. (Original) The method for screening of compounds according to claim 13, wherein said plurality of differing blocking agents comprise a cofactor and a regulatory agent solution.

15. (Original) The method for screening of compounds according to claim 1, wherein merging comprises application of electrostatic force.

16. (Original) The method for screening of compounds according to claim 1, wherein merging comprises drop deposition.

17. (Original) The method for screening of compounds according to claim 1, wherein merging comprises drop translation along a support.

18. (Original) The method for screening of compounds according to claim 1, wherein merging comprises fluid packet translation through not less than one channel.

19. (Original) The method for screening of compounds according to claim 1, wherein merging comprises continuous fluid translation through not less than one channel.

20. (Original) The method for screening of compounds according to claim 1, wherein merging comprises drop translation through not less than one channel.

21. (Original) The method for screening of compounds according to claim 7, wherein all said at least one preferred binding sites are blocked at said not less than one second location on the device.

22. (Original) The method for screening of compounds according to claim 13, wherein all said not less than one binding sites to be avoided are blocked at said not less than one first location on the device.

23. (Original) The method for screening of compounds according to claim 21, wherein all said preferred binding sites are blocked in said second location on the device and all said binding sites to be avoided are blocked in said first location on the device.

24. (Original) The method for screening of compounds according to claim 23, wherein alternate preferred binding sites are selected to be blocked in said second location on the device and alternate binding sites to be avoided are selected to be blocked in said first location of the device.

25. (Original) The method for screening of compounds according to claim 1, further comprising performing a preliminary screening for a surrogate substrate.

26. (Currently Amended) A method for screening of compounds for binding differentiation at various drug target binding sites, for use with a nanocalorimetric device

measuring the enthalpy of reaction for such binding, wherein said nanocalorimetric device includes thermal isolation regions, reference regions, and measurement regions, the method comprising:

depositing not less than one drop of a target compound within not less than one measurement region, wherein said target compound includes not less than one binding site of interest and not less than one binding site to be avoided;

depositing not less than one drop of a target compound within not less than one reference region, wherein said target compound includes not less than one binding site of interest and not less than one binding site to be avoided;

depositing not less than one drop of test compound within not less than one measurement region;

depositing not less than one drop of test compound within not less than one reference region;

merging said target compound with said test compound in the presence of at least one first blocking agent within the not less than one reference region;

merging said target compound with said test compound within the not less than one measurement region;

detecting a first heat of reaction for said merged target compound and said test compound in the presence of said at least one blocking agent within the not less than one reference region;

detecting a second heat of reaction for said merged target compound and said test compound within the not less than one measurement region; ~~and~~

comparing said heats of reaction for the not less than one reference region and the not less than one measurement region; and

determining a binding operation has occurred between said merged target compound and said test compound within the not less than one measurement region when the comparing step finds the second heat of reaction is greater than the first heat of reaction.

27. (Previously Presented) The method for screening of compounds for binding differentiation at various drug target binding sites according to claim 26, wherein said at

least one first blocking agent comprises a site-specific agent.

28. (Previously Presented) The method for screening of compounds for binding differentiation at various drug target binding sites according to claim 26, wherein said at least one first blocking agent comprises an excess of an enzyme specific substrate.

29. (Previously Presented) The method for screening of compounds for binding differentiation at various drug target binding sites according to claim 26, wherein said not less than one drop of a target compound comprises an enzyme target solution.

30. (Previously Presented) The method for screening of compounds for binding differentiation at various drug target binding sites according to claim 26, wherein said test ligand and said target compound are in the presence of at least one second blocking agent within said measurement region.

31. (Previously Presented) The method for screening of compounds for binding differentiation at various drug target binding sites according to claim 29, wherein said at least one second blocking agent comprises a cofactor solution and said at least one first blocking agent comprises an enzyme specific substrate solution.

32. (Previously Presented) The method for screening of compounds for binding differentiation at various drug target binding sites according to claim 29, wherein said first blocking agent comprises a plurality of blocking agents.

33. (Previously Presented) The method for screening of compounds for binding differentiation at various drug target binding sites according to claim 31, wherein said

plurality of blocking agents comprise enzyme specific substrate solution and regulatory agent solution.

34. (Previously Presented) The method for screening of compounds for binding differentiation at various drug target binding sites according to claim 32, wherein said plurality of blocking agents include an excess of enzyme specific substrate and a non-hydrolyzable nucleotide phosphate analog solution.

35. (Previously Presented) The method for screening of compounds for binding differentiation at various drug target binding sites according to claim 29, wherein said target compound comprises not less than one kinase target solution.

36. (Previously Presented) The method for screening of compounds for binding differentiation at various drug target binding sites according to claim 35, wherein said second blocking agent comprises an excess of ATP.

37. (Previously Presented)The method for screening of compounds for binding differentiation at various drug target binding sites according to claim 36, wherein said first blocking agent comprises a kinase-specific substrate.

38. (Previously Presented) The method for screening of compounds for binding differentiation at various drug target binding sites according to claim 32, wherein said at least one second blocking agent comprises a plurality of differing blocking agents.

39. (Previously Presented) The method for screening of compounds for binding differentiation at various drug target binding sites according to claim 26, wherein merging comprises application of electrostatic force.

40. (Previously Presented) The method for screening of compounds for binding differentiation at various drug target binding sites according to claim 26, further comprising performing a preliminary screening for a surrogate substrate.

41. (Previously Presented) The method for screening of compounds for binding differentiation at various drug target binding sites according to claim 27, wherein said drops have a drop size, said drop size ranging from approximately 100pL to approximately 100µL.

42. (Previously Presented) The method for screening of compounds for binding differentiation at various drug target binding sites according to claim 32, wherein all said at least one preferred binding sites are blocked at said not less than one reference region.

43. (Previously Presented) The method for screening of compounds for binding differentiation at various drug target binding sites according to claim 38, wherein all said not less than one binding sites to be avoided are blocked at said not less than one measurement region.

44. (Previously Presented) The method for screening of compounds according to claim 38, wherein said plurality of differing blocking agents comprise a cofactor and a regulatory agent solution.

45. (Previously Presented) The method for screening of compounds according to claim 42, wherein all said preferred binding sites are blocked in said reference region and all said binding sites to be avoided are blocked in said measurement region.

46. (Previously Presented) The method for screening of compounds according to claim 45, wherein alternate preferred binding sites are selected to be blocked in said reference region and alternate binding sites to be avoided are selected to be blocked in

said measurement region.

47. (Previously Presented) The method for screening of compounds for binding differentiation at various drug target binding sites according to claim 26, wherein merging comprises application of electric force.

48. (Original) The method for screening of compounds according to claim 1, wherein merging comprises application of electric force.

49. (Currently Amended) A method for screening of compounds for binding differentiation at various drug target binding sites, for use with a device measuring the enthalpy of reaction for such binding, wherein said device includes thermal isolation regions, reference regions, and measurement regions, the method comprising:

depositing not less than one drop of a target compound within not less than one measurement region, wherein said target compound includes not less than one binding site of interest and not less than one binding site to be avoided;

depositing not less than one drop of a target compound within not less than one reference region, wherein said target compound includes not less than one binding site of interest and not less than one binding site to be avoided;

depositing not less than one drop of test compound within not less than one measurement region;

depositing not less than one drop of test compound within not less than one reference region;

merging said target compound with said test compound in the presence of at least one first blocking agent within the not less than one reference region;

merging said target compound with said test compound within the not less than one measurement region;

detecting a first heat of reaction for said merged target compound and said test compound in the presence of said at least one blocking agent within the not less than one reference region;

detecting a second heat of reaction for said merged target compound and

said test compound within the not less than one measurement region; ~~and~~

comparing said heats of reaction for the not less than one reference region and the not less than one measurement region; and

determining a binding operation has occurred between said merged target compound and said test compound within the not less than one measurement region when the comparing step finds the second heat of reaction is greater than the first heat of reaction.

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